

# Rapid plasma reagin (RPR) card test

## *A screening method for treponemal disease*

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The specific FTA-ABS and TPI tests are too costly and time-consuming to be used as screening procedures for treponemal disease. The VDRL test detects reagin, the antibody amongst others produced in syphilis and yaws and also present in a wide variety of disorders giving the biological false positive reaction. The RPR card test (Portnoy, Garson, and Smith, 1957; Reed, 1965), like the VDRL test, uses cardiolipin antigen, but owing to the presence of added carbon particles flocculation in a reactive specimen can be read without the aid of a microscope; a proportion of biological false positive reactions can be expected, and the two tests therefore provide a screening procedure only.

The purpose of this investigation was to evaluate the RPR test in comparison with the VDRL, CWR, and RPCF tests, positive results being further investigated by the FTA-ABS and TPI tests.

### Materials, patients, and methods

Sera from 6,225 adult patients were tested.

Blood was obtained by venepuncture, centrifuged, and divided into two portions. On one the RPR card test was performed. The other was heated and the routine hospital screening tests (VDRL, RPCF, and CWR) were performed by a skilled laboratory technician. The RPR card test was performed by two technically inexperienced operators and by an experienced laboratory technician, all of whom learnt the acceptable technique and interpretation of results within 1 or 2 days. Qualitative tests were performed in batches of 70 to 90 a day (no isolated specimens are included).

The specimens comprised 4,800 from the VD clinic, 700 from antenatal clinics, 630 from the local male and female prison centres, and 95 from other in-patient sources, mostly from cases in which the possibility of latent syphilis was to be investigated.

The test requires a mechanical horizontal rotator, test cards and 'dispensstirs', antigen, and dropper. Cards are marked with ten circular test areas, and 0.05 ml. of serum or plasma (unheated) is dropped on to each test area from a disposable pipette ('dispensstir'). Each dispensstir is designed to expel a drop slightly in excess of 0.05 ml. to compensate for the amount of specimen retained during stirring. The serum is stirred and 1/60 ml. antigen is

dispensed using a needle and plastic dispenser. Specimen and antigen are mixed mechanically by rotation for 8 min. at 100 r.p.m. at room temperature. A plastic cover with an enclosed sponge, kept constantly wet, is laid over the cards when rotating, to ensure adequate humidification. Reactive specimens show agglutination of carbon particles from the antigen mixture, and these agglutinates are clearly visible with the naked eye against the white card. This 18 mm. circle card test was used as it is the test of choice when venous blood is employed and a large volume of serum or plasma is available as is the case in most clinical laboratories. The preparations, ten on each card, may be retained after drying and may be filed for reference. Control cards are available, each with reactive and non-reactive samples dried *in situ* and reconstituted with water. These gave consistent results. The minimally reactive samples required extended rotation times to show agglutination only when the room temperature was less than 65°F. We tested each new batch of antigen with a control card. For quantitative testing the above technique was used after diluting the serum with normal saline in doubling dilutions. In all cases in which the RPR card test was shown to be reactive and the VDRL non-reactive, sera were sent to the Venereal Disease Reference Laboratory where FTA-ABS, TPI, and VDRL tests were performed. When both RPR card tests and routine screening tests were reactive the sera were also examined by the Reference Laboratory. All reactive RPR results were compared with the standard screening test results and clinical findings as recorded by the physicians concerned in the various clinics, but at no time did the laboratory technician or those of us carrying out the RPR card test know each other's results. The final diagnosis in all cases sero-positive to reagin tests was made by consultant venereologists after full clinical assessment and consideration of results of the Reference Laboratory serum tests.

### Results

In a total of 6,225 sera tested, there were 540 specimens reactive by the RPR card test. Table I (overleaf) shows these results in comparison with the findings on the same sera screened by the VDRL test. The positive RPR card test and VDRL results were considered as false positives where there was failure of confirmation in the FTA-ABS and TPI results. There were 95 such cases with the RPR card test compared with 86 cases with the VDRL: in 65 of

these 181 sera, RPR and VDRL tests were both positive.

TABLE I *Results of 562 sera reactive in the RPR card test*

No. of sera	Test			Percentage
	VDRL	RPR	TPI+ FTA+	
407	+	+	+	72.5
65	+	+	-	11.5
21	+	-	-	3.7
30	-	+	-	5.3
1	+	-	+	0.2
38	-	+	+	6.8
562				100

There were 38 which were proved to be cases of treponemal disease (by positive results to the FTA-ABS or TPI tests in 37 and by darkfield microscopy in one) in which the RPR card test had proved positive but the VDRL test negative. These are presented in detail in Tables IIA and IIB. The results of serological tests in the sixteen cases listed in Table IIA were finally judged by venerologists to indicate residual seropositivity due to previous infection by yaws. Data concerning the 22 cases of syphilis are presented in detail in Table IIB; the

treatment status is given, lumbar puncture having been performed where necessary. Results in all 38 cases are summarized in Table III.

TABLE III *RPR positive, VDRL negative sera confirmed as cases of treponemal disease*

Diagnosis	No. of sera	Treatment	
		Yes	No
Previous yaws infection	16	10	6
<i>Treponema pallidum</i> infection	22	8	14
Primary	3	0	3
Secondary	2	0	2
Early latent	8	5	3
Latent	6	2	4
Late latent			
Late symptomatic	2	0	2
Congenital	1	1	0

Finally, 81 sera were examined quantitatively by the RPR card test and the results were compared with VDRL titres as reported by the Venereal Disease Reference Laboratory. The findings are presented in Table IV (opposite).

### Discussion

The RPR card test is rapid to perform and simple to read, giving clear-cut positive and negative results.

TABLE IIA *Details of findings in sixteen sera tested, comparing RPR card test with standard screening tests and reference laboratory results in patients with previous infection with yaws*

Sex	Screening			Reference Laboratory		Treatment
	RPR	VDRL	Reiter	VDRL	FTA and TPI	
M	+	-	-	-	+	0
M	W+	-	-	Neat	+	✓
M	++	-	-	Neat	+	0
M	W+	-	-	-	+	✓
F	+	-	-	-	+	✓
F	+	-	-	-	+	0
F	++	-	+	-	+	0
F	+	-	+	-	+	✓
F	W+	-	+	-	+	✓
M	VW+	-	-	-	+	✓
M	+	-	-	-	+	✓
F	+	-	+	-	+	✓
F	++	-	-	Neat	+	0
F	+	-	+	-	+	0
M	+	-	-	-	+	✓
M	+	-	+	-	+	✓

✓ = treated

0 = no treatment

TABLE IIB Details of findings in 22 syphilitic sera tested, comparing RPR card test with standard screening tests and reference laboratory tests, together with diagnosis

Sex	Screening tests			Reference Laboratory			Treatment	Syphilis diagnosed
	RPR	VDRL	Reiter	VDRL	FTA	TPI		
M	+	-	-	1/16	+	+	0	Early latent
F	VW+	-	+	-	+	+	✓	Congenital
M	+	-	-	-	+	+	0	Late (gumma)
M	++	-	-	Neat	+	+	✓	Early latent
F	W+	-	-	-	+	+	✓	Late latent
F	+	-	+	-	+	-	0	Primary
M	++	-	-	Microscopic	-	-	0	Secondary
M	W+	-	-	-	+	+	0	Early latent
M	+	-	-	-	+	+	✓	Early latent
M	++	-	+	-	+	+	0	Late symptomatic
F	+	-	+	-	+	+	✓	Early latent
F	+	-	+	-	+	+	✓	Early latent
F	W+	-	-	-	±	+	✓	Latent
F	+	-	Weak	-	?	+	0	Late latent
F	+	-	Weak	-	+	+	0	Late latent
M	+	-	+	-	+	+	✓	Early latent
M	+	-	-	-	+	-	0	Primary
M	+	-	+	-	+	+	0	Late latent
F	++	-	+	Neat	+	+	0	Secondary
F	++	-	+	-	+	-	0	Early primary
M	+	-	-	-	+	+	0	Early latent
M	++	-	+	-	+	+	0	Latent

✓ = treated      0 = no treatment

TABLE IV Serial dilutions of sera in 81 cases, to compare RPR card test with VDRL slide test

Titre	No. of sera	VDRL slide test titre							
		1	2	4	8	16	32	64	128
1	6	5	1						
2	13	4	8	1					
4	20	1	7	12					
8	15			5	5	4	1		
16	17			4	3	8	2		
32	5				1	4			
64	2				1	1			
128	2						1	1	
256	1							1	
Total	81	10	16	22	10	17	4	2	

The steps involved are listed in Table V and the contrast with the VDRL test shows the latter to be the more complex and time-consuming. A disposable

TABLE V Stage-by-stage comparison of methods

VDRL (11)	RPR (4)
1. Centrifuge	1. Centrifuge
2. Pipette 0.05 ml. serum to tube	2. —
3. Inactivate 30 min. at 56°C.	3. —
4. Pipette 0.05 ml. serum to slide	4. Pipette 0.05 ml. to card
5. Preparation of antigen	5. —
6. Add antigen; mix 4 min.	6. Add antigen; rotate 8 min.
7. Microscopic reading	7. Macroscopic reading
8. Clean slides	8. —
9. Clean pipettes	9. —
10. Number tubes	10. —
11. Wash tubes	11. —

kit, the use of unheated serum, and naked eye reading of results are the major factors responsible for the RPR test's economy. Throughout the testing period frequent checks on reproducibility were

maintained with excellent results. The speed and ease of the procedure were demonstrated in one timed series in which 100 sera were examined qualitatively in 29 minutes. From the clinician's viewpoint the ease and rapidity is such that it is reasonable to carry out isolated tests while the patient awaits results.

The sensitivity of the RPR card test over that of the VDRL is an argument in its favour: eleven of the cases of syphilis would have been undetected had screening been limited to the VDRL and RPCF tests, and in the three cases of primary syphilis the RPR and FTA-ABS tests had become reactive before the VDRL.

In using cardiolipin antigen, the RPR card test gives reactive results in cases other than those of treponemal disease in which reagin is produced. Of the 6,225 sera tested, 540 were reactive in the RPR and 95 of these were classed as giving false positive results because the FTA-ABS and TPI tests had proved negative. Venereologists responsible for the final diagnoses were of the opinion that the majority of these cases were due to the biological false positive phenomenon. In this group were cases of systemic lupus erythematosus, infective mononucleosis, virus pneumonia, chronic lymphatic leukaemia, and Behçet's disease, but most of the BFP sera were among those from the 700 specimens from antenatal clinics. A small number of false positive reactions, all of short duration, were unexplained. The degree to which the RPR card test is reactive in conditions provoking the BFP has not been adequately evaluated: a limited study in leprosy by Portnoy (1966) revealed no cases.

The requirements for screening tests are accuracy, speed, and economy: our experience has demonstrated these qualities in the RPR card test.

### Summary

The rapid plasma reagin (18 mm. circle) card test was evaluated as a screening test for treponemal

infection in the examination of 6,225 sera. Comparison was made with the VDRL and RPCF tests, and in positive cases sera were further examined by the FTA-ABS and TPI procedures. The RPR was found to be accurate, simple, and quick, as well as sensitive and of good reproducibility. It proved superior to the VDRL in that, among 446 sera from proven cases of treponemal infection, it gave positive results in 38 cases in which the VDRL had proved non-reactive: the RPR test gave only one false negative result.

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### References

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### Épreuve réaginique rapide sur carte avec le plasma Une méthode de dépistage pour les tréponématoses

#### SOMMAIRE

L'épreuve réaginique rapide sur carte (cercle de 18 mm.) avec le plasma (RPR) a été étudiée en tant qu'épreuve de dépistages des tréponématoses lors de l'examen de 6.225 sérums. Le RPR a été comparé au VDRL et au RPCF; les cas positifs furent examinés par le FTA-ABS et le TPI. Le RPR apparut précis, simple et rapide, aussi bien que sensible et de bonne reproductibilité. Il s'est montré supérieur au VDRL dans le fait que, pour 446 sérums provenant de cas certains d'infection tréponémique, il fut positif dans 38 cas pour lesquels le VDRL s'affirmait négatif; le RPR a donné seulement un faux résultat négatif.